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Altered Antibody Response to Influenza H1N1 Vaccine in Healthy Elderly People as Determined by HI, ELISA, and Neutralization Assay

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To determine the influence of ageing per se as well as of priming histories on the antibody response to influenza vaccination, haemagglutination inhibition (HI), ELISA IgG, IgA, IgM and neutralizing antibody titres were studied in 43 healthy young subjects (mean age 23 years) and 55 healthy elderly people (mean age 79 years). The HI and ELISA IgG responses to the A/Guizhou/54/89 strain (H3N2) for which both the young and the elderly had similar priming histories were equal. By contrast, the HI and IgG responses to A/Taiwan/1/86 (H1N1), where the priming histories were different, were lower in the elderly ($P < 0.05$). Influenza-specific IgA responses in the elderly tended to be higher for all vaccine strains. Influenza-specific postvaccination IgM titres were similar or tended to be higher in the elderly. A subgroup of elderly subjects (18%) who did not express HI activity to the A/Taiwan/1/86 (H1N1) vaccine strain, reacted in the HI assay with the closely related A/Singapore/6/86 (H1N1) strain. These elderly people, however, produced IgG antibodies which neutralized A/Taiwan/1/86 virus in vitro. It is concluded that the elderly are capable of mounting antibody responses similar to those observed in the young. Moreover, the observed age-related differences in antibody responses to H1N1 strains are probably not due to ageing of the immune system itself, but are determined by differences in priming histories. ***J. Med. Virol. 55:82–87, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: immunization; ageing; Senieur

INTRODUCTION

Studies on the humoral immune response to influenza vaccine in the elderly have yielded conflicting results. Some investigators observed declined antibody responses in elderly subjects, whereas others found similar or higher responses (for review see ref. [Beyer et al., 1989]). These divergent data may result from age-related differences in the priming histories of previous subtypes of influenza virus as well as concomitant disease [Remarque et al., 1996; Gross et al., 1989; Beyer et al., 1989]. Humoral immune responses to influenza vaccine are usually determined by HI, since homologous HI titres are correlated positively with resistance to infection [Longini et al., 1988; Potter et al., 1977; Wesselius-de Casparis et al., 1972; Hobson et al., 1972]. In some cases, however, HI may not detect influenza-specific antibodies which can be determined by ELISA [Okuno et al., 1993; Kida et al., 1994; Powers et al., 1992; Reuman et al., 1990]. This may be of importance, since the antibodies detected by ELISA can neutralize influenza virus in vivo [Powers et al., 1992; Reuman et al., 1990] as well as in vitro [Okuno et al., 1993; Kida et al., 1994]. Moreover, the distribution of these antibodies may depend on age [Powers et al., 1992].

The aim of the study was to determine whether ageing per se causes a decline in the humoral immune responses to influenza vaccine, taking care to avoid methodological errors as mentioned above. Differences in priming with previous subtypes of influenza are difficult to avoid, since age and previous exposure are closely intertwined. In this study previous exposure to H3N2 strains is assumed to be similar for both age

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Informed consent was obtained from all participants in the current study. Guidelines for human experimentation of the Leiden University Medical Centre were followed in the conduct of the clinical research. The study was approved by the Leiden University Medical Centre Ethics Committee.

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TABLE I. Characteristics of the Subjects Studied

	Young (n = 43)	Elderly (n = 55)
Age (mean ± SD)	23 ± 3	79 ± 3
Year of birth (range)	1964–1972	1906–1919
Females:Males (number)	23:20	31:24
Influenza vaccination ever (number %)	3 (7%)	15 (27%)

groups [McElhaney et al., 1993; Beyer et al., 1989]. For the H1N1 strains, the elderly are assumed to be primed by A-swine like H1N1 strains which circulated from 1918 to 1925, whereas the young are assumed to be primed by A/USSR/90/77-like H1N1 strains [McElhaney et al., 1993; Beyer et al., 1989].

MATERIALS AND METHODS

Subjects

The birth cohorts 1964 to 1972 and 1906 to 1919, respectively, were chosen to correspond with distinct influenza A priming periods [Beyer et al., 1989]. To minimize the influence of disease and medication, all young and elderly subjects were selected according to the Senieur protocol [Ligthart et al., 1984]. Once informed consent had been obtained, 43 young and 55 elderly persons were enrolled in the study. Their characteristics are presented in Table I. None of the subjects had received influenza vaccine over the previous two years, and all subjects were living independently in and around the city of Leiden, The Netherlands. The study was approved by the Medical Centre Ethics Committee of Leiden University.

Vaccines

The study was carried out in the autumn of 1990 and, according to WHO recommendations for the 1990/91 season, the tetravalent whole virus vaccine (Influvac(R), Duphar b.v., The Netherlands) contained 15 μg haemagglutinin each from A/Taiwan/1/86 (H1N1) and A/Guizhou/54/89 (H3N2) strains and 10 μg haemagglutinin each from B/Beijing/1/87 and B/Yamagata/16/88 strains. A single dose of vaccine was administered intramuscularly in the deltoid region. Blood samples were drawn prior to and 21 days after vaccination. After clotting and centrifugation, the sera were stored at −20°C.

Haemagglutination Inhibition

Haemagglutination inhibition (HI) titres were determined at the WHO National Influenza Centre for The Netherlands, Rotterdam, The Netherlands, following standard procedures using chicken erythrocytes and 4 haemagglutination units (HAU) of the appropriate virus [Masurel et al., 1981]. Before testing, the sera were treated with *Vibrio cholerae* filtrate and subsequently heated at 56°C to remove aspecific inhibitors [Masurel et al., 1981]. For the assessment of HI titres against influenza A whole viruses were used in the HI assay, whereas influenza B viruses were treated with ether to increase the sensitivity of the HI assay. Pre- and post-vaccination sera were titrated simultaneously and in duplicate against the vaccine strains A/Taiwan/1/86 (H1N1), A/Guizhou/54/89 (H3N2), B/Beijing/1/87, B/Yamagata/16/88 and some related influenza A strains, i.e., A/Singapore/6/86 (H1N1) and A/Beijing/353/89 (H3N2). Titres of serum samples without haemagglutination inhibiting activity at the lowest dilution (1:9) were recorded arbitrarily as 5. Seroconversion was defined as a four-fold or greater increase in HI titre following vaccination.

ELISA

Different isotypes of antibodies against the vaccine strains were determined by ELISA [Remarque et al., 1993]. Plates were coated overnight at 4°C with 100 μl per well of a 5 μg/ml concentration of the appropriate virus antigens in 0.1 M carbonate buffer pH 9.6 (donated by Solvay Duphar b.v., Weesp, The Netherlands). For the A/Taiwan/1/86 strain a subunit preparation was used, whereas whole virus preparations were used for the other strains. Remaining binding sites were blocked with 150 μl per well of 1% (w/v) bovine serum albumin (BSA) (Boseral, Organon Technica, Oss, The Netherlands) in 0.1 M carbonate buffer for 1 hr at 37°C. The plates were washed and 100 μl per well of the diluted serum samples were incubated for 2 hr at 37°C. After washing, the plates were incubated for 2 hr at 37°C with 100 μl per well Ig isotype-specific rabbit anti-human alkaline phosphatase conjugate (Dako, Glostrup, Denmark). After two washes the plates were incubated with substrate for 20′ at 37°C. The absorbance at 405 nm was read with a Bio-Rad model 3550 microplate reader (Bio-Rad Laboratories, Richmond, CA, USA). A dilution series of a reference serum pool was used on every plate and a standard curve was constructed. The reference serum was defined arbitrarily as containing one unit of antibody activity at the dilution yielding half maximal optical density (OD = 1.2). Hence, the amount of arbitrary units of a sample represents the reciprocal dilution at which an OD of 1.2 will be achieved. The antibody content of the samples was read from the linear part of the sigmoid standard curve.

Neutralizing Antibodies

Titres of neutralizing antibodies to the A/Taiwan/1/86 (H1N1) strain were determined with a microcytotoxicity test as described previously [van de Water et al., 1993]. Briefly, MDCK cells were incubated with virus and various serum dilutions. After 48 hr of incubation the medium was aspirated, the remaining living cells were washed twice and their proteins were stained with Amido black. Titres were expressed as the reciprocal serum dilution giving a 50% reduction of the cytotoxic effect. The A/Taiwan/1/86 live virus stocks used in the neutralization assay were obtained from Solvay Duphar b.v. Weesp, The Netherlands.

TABLE II. Geometric Mean Antibody Titres (CI 95%) to the Influenza A Vaccine Strains

		A/Guizhou/54/89 (H3N2)			A/Taiwan/1/86 (H1N1)		
		Young (n = 43)		Elderly (n = 55)	Young (n = 43)		Elderly (n = 55)
HI	Day 0	21 (14–32)		17 (12–25)	6 (5–7)		5 (5–6)
	Day 21	246 (187–324)		325 (222–476)	136 (99–187)	*	45 (29–71)
IgG	Day 0	839 (653–1078)		816 (638–1044)	490 (399–602)		511 (426–621)
	Day 21	2483 (1965–3138)		2251 (1169–3036)	2309 (1838–2901)	*	1551 (1247–1928)
IgA	Day 0	36 (29–46)		42 (32–56)	26 (20–34)		26 (19–34)
	Day 21	119 (89–160)	*	231 (158–336)	222 (158–321)		265 (176–397)
IgM	Day 0	263 (206–337)	*	81 (56–117)	30 (24–37)	*	17 (14–22)
	Day 21	508 (413–625)		380 (250–577)	184 (136–248)		188 (122–289)

*Denotes statistically significant difference ($P < 0.05$; ANCOVA) between the age groups.

Statistical Analysis

Statistical analysis on log2-transformed titres was carried out with the SPSS(TM) for Windows(TM) version 6.1 (SPSS, Chicago, IL). Evaluation of postvaccination titres was performed using analysis of covariance (Ancova), with prevaccination titre as covariate and age-group as explanatory variable. Differences in proportions were analysed by the χ^2 test.

RESULTS

H3N2 Strains

Prevaccination HI, IgG and IgA titres to the A/Guizhou/54/89 (H3N2) strain were similar for both age-groups, whereas IgM prevaccination titres were higher in the young as compared to the elderly (Table II). The HI, IgG and IgM responses to the A/Guizhou/54/89 (H3N2) vaccine strain were similar in both age-groups, whereas IgA responses were higher in the elderly ($P = 0.01$; ANCOVA, Table II). The HI responses to the related A/Beijing/353/89 (H3N2) strain were higher in the elderly (postvaccination HI titres 168, CI 95% 130 to 216 and 276, CI 95% 174 to 439, respectively for the young and elderly, $P < 0.05$; ANCOVA).

H1N1 Strains

Prevaccination HI, IgG and IgA titres to the A/Taiwan/1/86 (H1N1) strain did not differ for the two age-groups, but IgM titres were higher in the young (Table II). The HI and IgG responses to the A/Taiwan/1/86 (H1N1) strain were lower in the elderly than they were in the young ($P \leqslant 0.01$; ANCOVA, Table II). Postvaccination IgA and IgM titres were similar for both age-groups. In contrast, pre- and postvaccination HI titres to the A/Singapore/6/86 (H1N1) strain did not differ between the two age-groups (303, CI 95% 227 to 404 and 285 CI 95% 169 to 479, respectively, for the young and the elderly). This is a rather unexpected finding because the antibody response to the closely related H1N1 vaccine strain [Cox et al., 1989] was lower in the elderly. Therefore we compared HI seroconversions to both H1N1 strains were compared.

HI Seroconversions to H1N1 Strains

According to the seroconversion pattern to the H1N1 strains, four groups could be distinguished (Table III).

TABLE III. HI Seroconversion to H1N1 Strains in Groups of Young and Elderly Individuals

Seroconversion to			
A/Taiwan/1/86	A/Singapore/6/86	Young (n = 43)	Elderly (n = 55)
No	No	2 (5%)*	7 (13%)
Yes	No	3 (7%)	7 (13%)
Yes	Yes	37 (86%)	31 (56%)
No	Yes	1 (2%)	10 (18%)

The distribution of the numbers of young and elderly over these four groups differed significantly ($X^2 = 11.0$, 3 *df*, $P = 0.01$). *Figures in parenthesis are percentages.

The majority of the young (86%) seroconverted to both H1N1 strains, whereas only 56% of the elderly seroconverted to both H1N1 strains (Table III). The percentage of non-responders to both H1N1 strains was 5% in the young as compared to 13% in the elderly (Table III). Only a minority of the young (7% and 2%, respectively) seroconverted to either A/Taiwan/1/86 or A/Singapore/6/86 alone, as compared to 13% and 18%, respectively, of the elderly (Table III).

Since HI data concerning the antibody response to closely related H1N1 strains were conflicting, especially for a relatively large number of the elderly people, additional neutralization experiments were carried out. HI and neutralizing titres of the elderly responding with HI antibody to one of the two H1N1 strains under investigation were compared with random samples of the young (n = 12), and elderly (n = 20) responding to both H1N1 strains. In general, there was good agreement between HI and neutralization titres to A/Taiwan/1/86 for both the young and elderly (Fig. 1). However, the elderly who had HI antibody responses to A/Singapore/6/86, but not to A/Taiwan/1/86 (triangles n = 10), had an average neutralization titre to the A/Taiwan/1/86 virus of 80 (CI 95% 47 to 137). These neutralization titres were roughly similar to those in the elderly with HI antibody responses to A/Taiwan/1/86 only (circles, n = 7, average neutralization titre 121, CI 95% 61 to 241).

In addition, the elderly who did not have HI antibody responses to A/Taiwan/1/86, but had responses to A/Singapore/6/86, had ELISA IgG titres to A/Taiwan/1/86 that were similar to the elderly with responses to A/Taiwan/1/86 only (viz. 1155, CI 95% 792 to 1684 versus

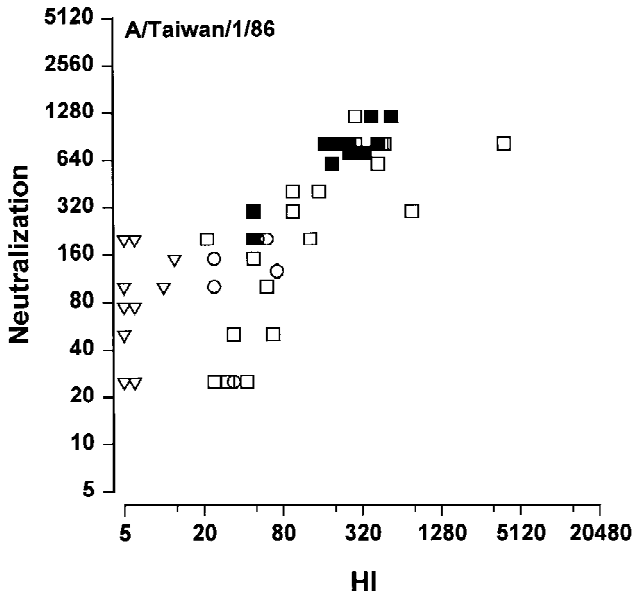

Fig. 1. Relation between HI and neutralizing titres to A/Taiwan/1/86, according to age and seroconversion patterns to the A/Taiwan/1/86 and A/Singapore/6/86 H1N1 strains. ■ Young responding to both strains (N = 12). □ Elderly responding to both strains (N = 20). ○ Elderly responding to A/Taiwan/1/86 only (N = 7) and ▽ elderly responding to A/Singapore/6/86 only (N = 10).

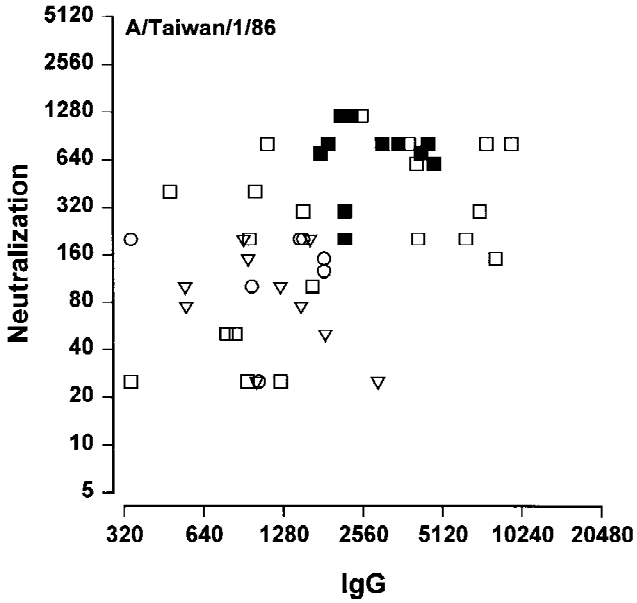

Fig. 2. Relation between IgG and neutralizing titres to A/Taiwan/1/86, according to age and seroconversion patterns to the A/Taiwan/1/86 and A/Singapore/6/86 H1N1 strains. ■ Young responding to both strains (N = 12). □ Elderly responding to both strains (N = 20). ○ Elderly responding to A/Taiwan/1/86 only (N = 7) and ▽ elderly responding to A/Singapore/6/86 only (N = 10).

1145, CI 95% 663 to 1977). Consequently, IgG titres to A/Taiwan/1/86 did not correlate with homologous HI titres. Nine out of 10 of the elderly who did not have an adequate HI response to A/Taiwan/1/86, but had an adequate HI response to A/Singapore/6/86, also had IgG antibodies to A/Taiwan/1/86 which neutralized the homologous virus (Fig. 2). Thus, A/Taiwan/1/86-specific IgG antibodies with neutralizing capacity were present, but these antibodies were not detected in homologous HI.

B-strains

Prevaccination IgG titres to the B/Beijing/1/87 strain were higher in the young than in the elderly. Postvaccination HI, IgG, IgA and IgM titres to B/Beijing/1/87 were similar for both age-groups. Prevaccination titres to the B/Yamagata/16/88 strain were similar for both age-groups. HI and IgG responses to the B/Yamagata/16/88 strain were lower in the elderly than in the young (P values $\leqslant 0.01$; ANCOVA)(Table IV). IgA responses to B/Yamagata/16/88 were similar for both age-groups, whereas IgM responses to B/Yamagata/16/88 were higher in the elderly ($P < 0.001$; ANCOVA, Table IV).

DISCUSSION

Lower HI antibody levels to the H3N2 vaccine component in the elderly were not found in this or in earlier studies [Palache et al., 1993; Glathe et al., 1993; Powers and Belshe, 1993; McElhaney et al., 1993; Zei et al., 1991; Iorio et al., 1989]. The antibody response to the H3N2 vaccine strain demonstrates that elderly subjects are capable of mounting antibody responses similar to those in young subjects. Moreover, postvaccination IgA titres to the H3N2 vaccine strain were higher in the elderly, as was observed previously [Remarque et al., 1993].

Under the assumption that immune function deteriorates with age, defects are expected to occur for many antigens. The healthy elderly subjects investigated in this study showed declined responses to certain vaccine strains only. In elderly subjects with various underlying conditions, however, responses to all vaccine strains were lower [Remarque et al., 1996]. Hence, these and other data from our group [Nijhuis et al., 1994; de Greef et al., 1992] do not provide evidence for a general decline in immune capacity in healthy elderly people. When decreased responses to several antigens are observed, this is probably due to poor health status [Remarque et al., 1996].

The poor antibody response to the A/Taiwan/1/86 (H1N1) strain observed in the elderly is in agreement with others [Powers and Belshe, 1993; McElhaney et al., 1993; Palache et al., 1993; Zei et al., 1991], although sometimes similar responses as in young subjects were observed [Glathe et al., 1993; Iorio et al., 1989]. The age-related difference in antibody specificity to closely related H1N1 strains is in accordance with results obtained in subjects with different priming backgrounds [Pyhälä et al., 1993]. Moreover, in that study, HI responses to the A/Taiwan/1/86 strain were also lower in the older subjects [Pyhälä et al., 1993]. However, the group designated as older in this latter study ranged from 35 to 60 years of age [Pyhälä et al., 1993]. Hence, age-related differences in the antibody response to closely related H1N1 strains as observed in the present study may not be due to ageing of the im-

TABLE IV. Geometric Mean Antibody Titers (CI 95%) to the Influenza B Vaccine Strains

		B/Beijing/1/87			B/Yamagata/16/88		
		Young (n = 43)		Elderly (n = 55)	Young (n = 43)		Elderly (n = 55)
HI	Day 0	20 (12–34)		14 (9–21)	9 (6–12)		6 (5–7)
	Day 21	290 (203–415)		177 (115–273)	232 (158–339)	*	80 (56–114)
IgG	Day 0	1280 (939–1744)	*	746 (515–1079)	858 (670–1098)		742 (581–947)
	Day 21	5007 (3819–6565)		2827 (1217–3775)	3490 (2784–4375)	*	1908 (1508–2415)
IgA	Day 0	31 (23–42)		32 (24–42)	30 (22–41)		39 (30–51)
	Day 21	142 (100–201)		186 (137–252)	145 (100–210)		213 (159–285)
IgM	Day 0	19 (16–23)		14 (12–17)	31 (18–53)		29 (20–40)
	Day 21	218 (150–315)		219 (141–342)	60 (29–121)	*	152 (83–279)

*Denotes statistically significant difference ($P > 0.05$; ANCOVA) between the age groups.

mune system but rather to differences in priming background of the subjects studied. Thus the lower HI and IgG responses to A/Taiwan/1/86 observed in the elderly are probably due to priming differences rather than to immunosenescence [McElhaney et al., 1993].

The relative insensitivity in the HI assay to detect antibodies to the A/Taiwan/1/86 strain is probably a peculiarity of this strain [Pyhälä et al., 1993]. However, it enabled us to detect age-related differences in the specificity of the antibodies induced by vaccination with a H1N1 strain. In about 15% of the elderly individuals the antibodies elicited by the H1N1 vaccine antigen bound to the homologous antigen by ELISA, neutralized viral infectivity in vitro, but did not inhibit haemagglutination by the homologous strain. This coincides with the observation that the elderly are at low risk of medically attended H1N1 infections [Glezen et al., 1991]. By contrast, antibody responses of this type were observed in less than 5% of the young subjects studied. Conversely, differences in the physical forms of the antigens or antigenic determinants of the antigens employed may provide an alternative explanation for the differences in the HI titres to the H1N1 strains. However, if this is true, a similar trend in the antibody responses of both age-groups would have been expected.

For the B strains, sometimes similar and sometimes lower responses were observed in the elderly as compared to the young, which is in agreement with others [Palache et al., 1993; Powers and Belshe, 1993; McElhaney et al., 1993; Glathe et al., 1993; Iorio et al., 1989; Zei et al., 1991]. The two B strains contained in the vaccine belong to antigenically distinct evolutionary lineages [Pyhälä et al., 1992; Kinnunen et al., 1992], with B/Beijing/1/87-like strains being the prevalent types before 1989 [Pyhälä et al., 1992]. Thus immunologic memory is assumed to be present for B/Beijing/1/87 and, due to a shorter circulation time in the population, to a lesser degree for the B/Yamagata/16/88 strain. Hence the response to the B/Beijing/1/87 strain is probably a recall response, whereas the response to the B/Yamagata/16/88 strain is, at least partially, a primary response. Therefore, the lower HI and ELISA IgG response to the B/Yamagata/16/88 strain in the elderly may be due to a reduced capacity to respond to novel, strain-specific antigenic determinants. This is supported by the observation that repeated influenza vaccinations improve antibody responses to B strains of B/Yamagata-like strains in the elderly [de Bruijn et al., 1997].

In conclusion, it was found that healthy elderly people were able to mount similar antibody responses to influenza vaccine strains to healthy young subjects. For certain strains antibody responses were lower in the elderly as compared to young subjects. These strain-related differences in the antibody response may be due to differences in priming histories, rather than to ageing of the immune system.

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